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Polysaccharides as Antiviral Agents: Antiviral Activity of Carrageenan

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A number of polysaccharides showed good antiviral activity against several animal viruses. At 5 μg/ml, carrageenan prevented the cell monolayer from destruction by herpes simplex virus type 1 (HSV-1) growth. At 10 μg/ml, carrageenan reduced the formation of new infectious HSV-1 by almost five logs. No cytotoxic effects were detected with concentrations of carrageenan up to 200 μg/ml. When 10 μg of carrageenan per ml was added at the beginning of HSV-1 infection of HeLa cells, there was potent inhibition of viral protein synthesis, and the cells continued synthesizing cellular proteins. This did not occur if carrageenan was added 1 h after HSV-1 infection. The use of [^{35}S]methionine-labeled virions to analyze the entry of HSV-1 or Semliki Forest virions into cells indicated that carrageenan had no effect on virus attachment or virus entry. Moreover, carrageenan did not block the early permeabilization of cells to the toxic protein alpha-sarcin. These results suggest that this sulfated polysaccharide inhibits a step in virus replication subsequent to viral internalization but prior to the onset of late viral protein synthesis.

Polysaccharides are a complex group of biological molecules (2) known to affect the growth of animal viruses (19). The routine use of the plaque assay method to titrate animal viruses soon led to the discovery of inhibitory components in the agar overlay (20). These components were identified as sulfated polysaccharides. Plaque production by encephalomyocarditis virus was inhibited in normal agar, whereas a mutant strain was resistant (22). Addition of a polycation such as DEAE-dextran counteracted the inhibitory action of the negatively charged inhibitor (22). Similar findings were described for poliovirus (23) and coxsackievirus B4 (3). However, subsequent studies showed that the enhancement of viral plaques by polycations might not be related to their interaction with sulfated polysaccharides present in the agar (25). Heparin also showed a potent inhibitory effect on herpesvirus replication (16, 24). Since heparin is a polyanionic molecule, it was suggested that it would interact with the positive charges present on the virus or on the cell surface and inhibit virus attachment (20). In agreement with this idea, heparin was only active against virus growth when present during the early stages of viral replication (16). Prevention of viral attachment by polyanions was reported by several laboratories (11, 20). However, the method used to measure virus absorption was crude (4, 11, 13), and the results obtained require careful interpretation.

Other therapeutically important effects of polysaccharides have also been reported. For instance, heparin promoted tumor regression in mice (10). In addition, a number of natural and synthetic polyanions were also effective inducers of interferon both in vivo and in vitro (5, 6, 14). Dextran sulfate also prolonged the incubation period of the scrapie agent (7). The antiviral effects of some of these compounds in animals may be a consequence of macrophage activation or interferon induction or both (5).

In a screening for antiherpes agents with a number of natural substances, we found that carrageenan had a potent inhibitory effect against herpes simplex virus type 1 (HSV-1) (1). We have now analyzed in more detail the antiviral properties of this polysaccharide.

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MATERIALS AND METHODS

Cells and viruses. Human HeLa cells, baby hamster kidney cells clone 21, mouse fibroblast cells (L929), and green monkey kidney cells (Vero) were grown in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum and containing antibiotics (10,000 IU of penicillin and 50 mg of streptomycin per ml). The maintenance medium contained only 2% newborn calf serum.

Adenovirus type 5, poliovirus type 1, and vaccinia virus were grown on HeLa cells. Encephalomyocarditis virus was grown on L929 cells. Semliki Forest virus, vesicular stomatitis virus Indiana strain, HSV-1 KOS strain, HSV-2, measles virus, and African swine fever virus were grown on Vero cells. Most of these viruses were originally purchased from the American Type Culture Collection, Rockville, Md. Otherwise, HSV-1 was kindly given to us by E. de Clercq (Louvain, Belgium), vaccinia virus was given by M. Esteban (New York, N.Y.), and African swine fever virus was given by E. Tabarés (Madrid, Spain). The virus concentration was estimated by plaque assay on the same cell line as that on which the virus was grown.

Source of polysaccharides. Polysaccharides were purchased from Sigma Chemical Co., St. Louis, Mo., except agar, which was from Oxoid Ltd., London, England. Alpha-sarcin, a toxic protein (molecular weight, 16,800) produced by *Aspergillus giganteus*, was a generous gift from D. M. Schuurmans (Department of Public Health, Lansing, Mich.).

Plaque assay. Confluent cell monolayers were grown on P60 plates and incubated with 0.5 ml of serial 10-fold dilutions of virus in phosphate-buffered saline supplemented with 0.5% calf serum. After an adsorption period of 1 h at 37°C, the inoculum was removed and 5 ml of an overlay of Dulbecco modified Eagle medium containing 0.6% agar and 2% calf serum was added (adenovirus-incubated cells were overlaid with Dulbecco modified Eagle medium supplemented with 3% fetal calf serum, 25 mM $MgCl_2$, 2.5 mM

arginine, 0.1 mg of DEAE-dextran per ml, and 0.6% agar). Cell monolayers were incubated at 37°C in a humidified atmosphere of 6% CO_2 in air for several days until a cytopathic effect was seen. The overlay was removed, the cell monolayer was precipitated with 5% trichloroacetic acid, and plaques were counted.

Estimation of cytopathic effect. Monolayers of HeLa cells were infected with virus at low multiplicity (0.1 to 0.4 PFU per cell) in the presence of the indicated concentrations of each polysaccharide. After several virus replication rounds (24 or 48 h of incubation at 37°C), the cytopathic effects in infected and uninfected cells were examined under a phase-contrast microscope. To gain further insight into the cytotoxic effects of these compounds, the level of translation was determined by 1-h incubation with 0.2 μCi of [^{35}S]methionine (1,100 Ci/mmol; The Radiochemical Centre, Amersham, England). Radioactivity of the trichloroacetic acid-precipitable material was measured in an Intertechnique liquid scintillation spectrometer. Each experimental measurement was carried out in duplicate.

Measurement of production of HSV-1 infectious units. HeLa cells were grown in 24-well plates and infected with HSV-1 at 0, 5, or 10 PFU per cell in the presence of various concentrations of polysaccharide to analyze the reduction of infectious units in several or one single cycle of replication. After 48 or 24 h of incubation at 37°C, the medium and the infected cells were collected and cells were disrupted by three freeze-thaw cycles. The infectious viruses produced were estimated by the standard plaque assay.

Effect of polysaccharide on cell proliferation. An average of 3×10^4 HeLa cells per well were seeded in 24-well plates with Dulbecco modified Eagle medium supplemented with 10% newborn calf serum. Cells were infected with HSV-1 at a multiplicity of 10 in the presence of 100 μg of polysaccharide per ml, or were not infected, and were incubated at 37°C for 4 days. Each day, cells of four wells were trypsinized and counted with a Neubauer hemacytometer, and the mean value was calculated.

Measurement of protein synthesis. A 0.2-ml per well amount of methionine-free medium containing 0.2 μCi of [^{35}S]methionine was added to cells grown on 24-well dishes. The cell monolayer was incubated at 37°C for 1 h and the medium was then removed. The cells were fixed with 5% trichloroacetic acid and washed with ethanol. The fixed cell monolayer was dried and suspended in 0.2 ml of 0.1 N NaOH–1% sodium dodecyl sulfate, and incorporated label was measured as above.

Analysis of proteins by polyacrylamide gel electrophoresis. HeLa cells grown on 24-well plates were incubated in methionine-free medium in the presence of 5 μCi of [^{35}S]methionine (1,100 Ci/mmol; The Radiochemical Centre) per ml for the indicated times. After incubation, the medium was removed and the cell monolayer was washed with phosphate-buffered saline. The cells were dissolved in 0.1 ml of sample buffer (62.5 mM Tris [pH 6.8], 2% sodium dodecyl sulfate, 0.1 M dithiothreitol, 17% glycerol), sonicated, and heated to 90°C for 10 min. Samples of 10 μl were analyzed by polyacrylamide gel electrophoresis, using 15% acrylamide gels. The 20-cm-long gels were run overnight at 85 V, and fluorography was performed with 2,5-diphenyloxazole (20%, wt/wt). The dried gels were exposed, using XS-5 X-ray films (Eastman Kodak Co., Rochester, N.Y.).

Radiolabeling and purification of virions. HeLa cells were grown on 100-mm-diameter dishes. The cell monolayer was infected with virus at a multiplicity of 10 in Dulbecco modified Eagle medium supplemented with 2% newborn calf serum. At 9 h postinfection the medium was replaced with 2 ml of fresh methionine-free medium and 100 μCi of [^{35}S]methionine was added. At 24 h postinfection, the cells were harvested and disrupted by freezing and thawing. The cellular extracts were centrifuged at 10,000 rpm for 15 min in an SS-34 Sorvall rotor. The supernatants were layered onto 3 ml of 50 mM Tris (pH 7.4) with 0.85% NaCl and 30% sucrose and centrifuged at 40,000 rpm for 3 h in a TY-65 Sorvall rotor. Pellets were suspended in 0.5 ml of Dulbecco modified Eagle medium supplemented with 2% newborn calf serum.

Measurement of the entry of virus particles into cells. HeLa cells grown on 35-mm petri dishes were infected with HSV-1 labeled with [^{35}S]methionine, purified as described above, and incubated at 37°C. At the indicated times, cell monolayers were washed with saline-phosphate buffer and treated with 1 ml of saline-phosphate buffer containing 50 mg of proteinase K (Merck AG) per ml for 15 min at 4°C. The cells were then washed with saline-phosphate buffer, and pellets were precipitated with 5% trichloroacetic acid. Radioactivity retained in GF/C filters was determined in a liquid scintillation counter (Intertechnique).

TABLE 1. Effect of polysaccharides on several animal viruses

Polysaccharide	Virus[a]	CPE_{50} (μg/ml)[b]
Agar	HSV-1	>200
Alginic acid	HSV-1	20
Alginic acid (sodium salt)	HSV-1	50
Carboxymethyl cellulose	HSV-1	>200
Cellulose	HSV-1	>200
Chitin	HSV-1	>200
Chondroitin sulfate	HSV-1	>200
Dextran 500,000	HSV-1	>200
Heparin	HSV-1	10
Inulin	HSV-1	>200
Starch	HSV-1	>200
Iota-carrageenan	Ad5	>200
	ASF	10
	EMC	10
	HSV-1	2
	HSV-2	10
	Measles	>200
	Polio, type 1	>200
	SFV	10
	Vaccinia	10
	VSV	>200
Dextran sulfate 5000	ASF	20
	EMC	5
	HSV-1	5
	Polio, type 1	>200
	SFV	>200
	VSV	>200
Dextran sulfate 500,000	HSV-1	1
	Polio, type 1	>200
	VSV	10

[a] Ad5, Adenovirus type 5; ASF, African swine fever; SFV, Semliki Forest virus; VSV, vesicular stomatitis virus; EMC, encephalomyocarditis.

[b] CPE_{50}, Concentration of compound that conferred 50% protection of the cytopathic effect. Each value presented was taken from one experiment, where 10 different concentrations of polysaccharide were tested in replicate monolayers. None of the polysaccharides screened showed a cytotoxic effect even at 200 μg/ml.

RESULTS

Antiviral effect of polysaccharides against several animal viruses. The effects of different polysaccharides on the growth of several animal viruses are shown in Table 1. The most active polysaccharides were those that possess sulfated groups, such as carrageenan, heparin, and dextran sulfate, although other nonsulfated compounds such as alginic acid were also active. On the other hand, chondroitin sulfate (Sigma Chemical Co.) did not protect cells from virus growth, perhaps because the degree of sulfation of this polysaccharide is low. Not only were high-molecular-weight polysaccharides active, but oligosaccharides such as dextran sulfate (molecular weight, 5,000) also showed good antiviral activity. As regards the spectrum of action of carrageenan, it was active against some enveloped viruses such as HSV-1, HSV-2, and Semliki Forest, vaccinia, and swine fever viruses. It was without effect against vesicular stomatitis virus and measles virus. It also had an inhibitory effect against naked viruses like encephalomyocarditis virus, but it was devoid of any activity against poliovirus or adenovirus. These results are more clearly observed in Fig. 1, where the cytopathic effect and the translation capacity of cells treated with carrageenan are analyzed. In general, there is agreement between the results obtained on the prevention of the cytopathic effect and the prevention of the translation capacity of the cell monolayer. It is noteworthy that 200 μg of carrageenan per ml had no deleterious effects on uninfected cells as observed by phase-contrast microscopy or by measuring the capacity of the cells to incorporate [^{35}S]methionine into protein after 2 days of incubation with the polysaccharide. On the other hand, 10 μg of carrageenan per ml fully protected against the destruction of the cell monolayer by HSV-1 growth. More detailed studies with lower concentrations of carrageenan have shown that 50% protection of the cytopathic effect against HSV-1 corresponds to 2 μg/ml. Keeping in mind that the average molecular weight of carrageenan is 500,000, this gives an average concentration of 4×10^{-9} M. The antiviral index for carrageenan is above 100 as defined by the concentration of the compound that would produce a 50% inhibition of cell growth (>200 μg/ml) divided by the concentration that confers 50% protection of the cytopathic effect (<2 μg/ml).

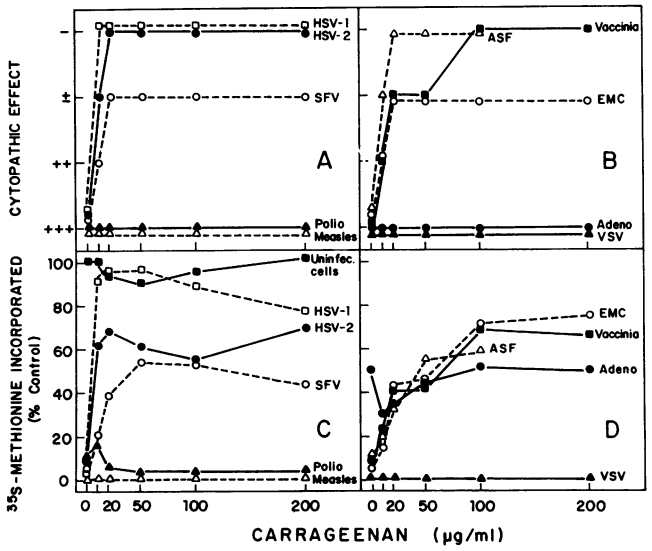

FIG. 1. Protection of the cytopathic effect of HeLa cells infected with different viruses in the presence of the indicated concentrations of carrageenan (A and B). Protein synthesis was measured in virus-infected cells and in control uninfected cells (C and D). SFV, Semliki Forest virus; EMC, encephalomyocarditis virus; VSV, vesicular stomatitis virus; ASF, African swine fever virus.

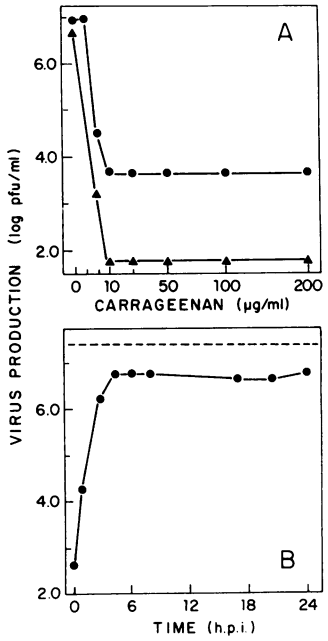

FIG. 2. Effect of carrageenan on production of infectious virus. (A) Production of PFU of HSV-1 in infected HeLa cells treated with carrageenan from the beginning of infection. Cells were collected and PFU were evaluated by the standard plaque assay on Vero cells. Symbols: ●, PFU production during a single round of replication at a multiplicity of 10 PFU per cell; ▲, PFU production during several rounds of replication at a multiplicity of 0.5 PFU per cell. (B) Time dependence of carrageenan (100 μg/ml) addition to HSV-1-infected HeLa cells. Production of PFU was evaluated by the standard plaque assay on Vero cells. p.i., Postinfection.

Effect of carrageenan on HSV-1 and cell growth. The reduction of HSV-1 growth by carrageenan is shown in Fig. 2. As little as 10 μg of carrageenan per ml profoundly inhibits the production of infectious HSV-1 either in a single cycle or

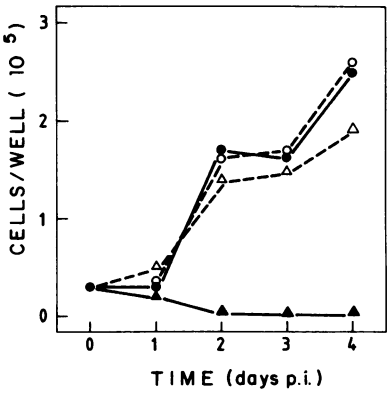

FIG. 3. Effect of carrageenan on cell proliferation. HeLa cells were seeded at a concentration of 3×10^4 cells per well. They were mock infected and incubated at 37°C in the absence (●) or presence (○) of 100 μg of carrageenan per ml, or they were infected with HSV-1 at a multiplicity of 10 PFU per cell in the absence (▲) or presence (△) of 100 μg of carrageenan per ml. p.i., Postinfection.

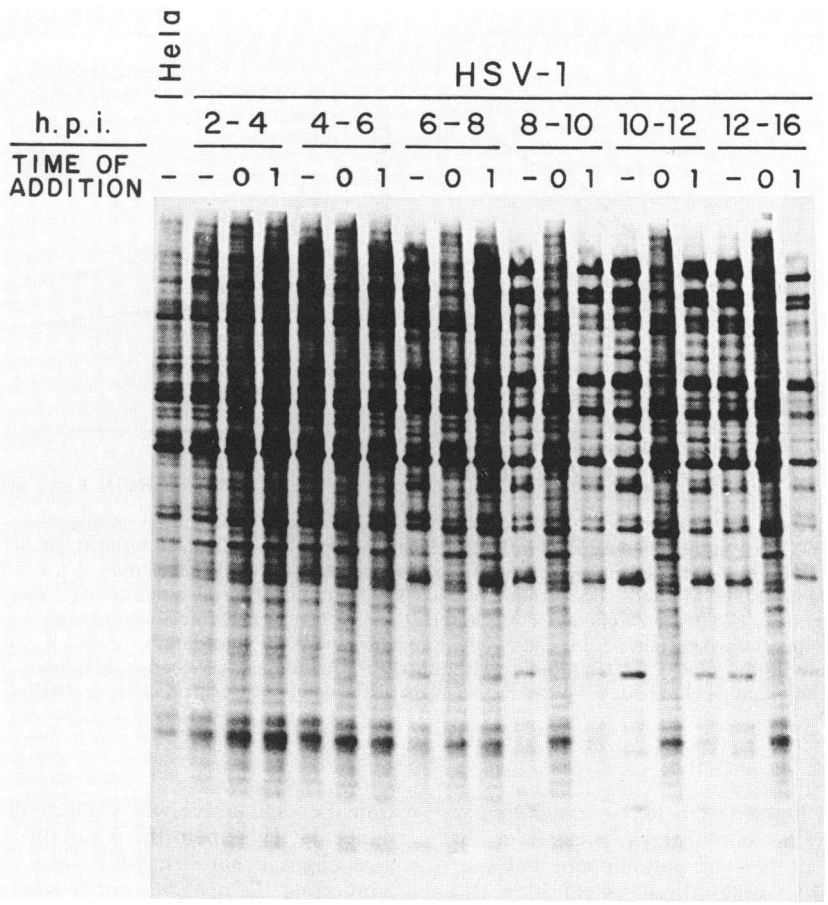

FIG. 4. Polyacrylamide gel electrophoresis of the proteins synthesized in HSV-1-infected HeLa cells at several times postinfection (p.i.) in the absence (−) or presence of 100 μg of carrageenan per ml added from the beginning of infection (0) or 1 h postinfection (1).

in several rounds of replication (Fig. 2A). The time of addition of carrageenan is very important, because the compound is most active when added at the very early stages of virus growth and has only a marginal effect when added after h 4 of HSV-1 infection (Fig. 2B). We have also observed that pretreatment of cells with carrageenan does not confer protection against HSV-1 and does not show direct virucidal effects (results not shown).

As described above, carrageenan has no effect on cell morphology or on the capacity of the cells to synthesize proteins after 2 days of incubation (Fig. 1). To further test the toxicity of carrageenan to culture cells, the inhibition of cell proliferation was measured. Figure 3 shows that HeLa cells seeded at low concentration grow at control levels in the presence of 100 μg of carrageenan per ml. Moreover, even HSV-1-infected cells are able to proliferate in the presence of the polysaccharide, suggesting that carrageenan allows HSV-1-infected cells to survive (Fig. 3).

Antiherpes mechanism of action of carrageenan. To ascertain the effect of carrageenan on protein synthesis in HSV-1-infected cells, the experiment shown in Fig. 4 was done. HeLa cells were infected with HSV-1 and carrageenan was added immediately or 1 h after HSV-1 infection. Protein synthesis was then analyzed at various times after infection. When carrageenan was present from the very beginning of infection, viral proteins were not detected, whereas if carrageenan was added 1 h after HSV-1 infection late viral proteins were apparent. These results demonstrate that carrageenan must be present from early times to exert its antiherpes activity and that the step blocked by this polysaccharide in viral replication is prior to the synthesis of late viral proteins. The concentration dependence of carrageenan on the inhibition of viral protein synthesis showed that some viral proteins still appeared at 5 μg of carrageenan per ml, and virtually no viral proteins were detected at concentrations above 20 μg/ml (results not shown). Therefore, we routinely used 100 μg of carrageenan per ml in our subsequent experiments.

Previous studies from other laboratories indicated that sulfated polysaccharides inhibited virus attachment to the cell surface (20). To investigate this possibility, HSV-1 virions were labeled with [^{35}S]methionine and virus entry into cells was measured in the absence or presence of carrageenan. To our surprise, no inhibition of virus internalization was observed even in the presence of 100 μg of carrageenan per ml (Fig. 5A), suggesting that this polysaccharide blocked a step in virus replication subsequent to virus attachment and entry. This result is in good agreement with our finding that increased internalization of [^{3}H]heparin occurs during HSV-1 entry into cells (González and Carrasco, unpublished results). Therefore, it seems reasonable to suggest that viral particles and the polysaccharide are cointernalized in endosomes. The polymer may inhibit a step in virus replication occurring between virus release from pinocytotic vesicles and the synthesis of late proteins.

During virus entry the infected cells become permeabil-

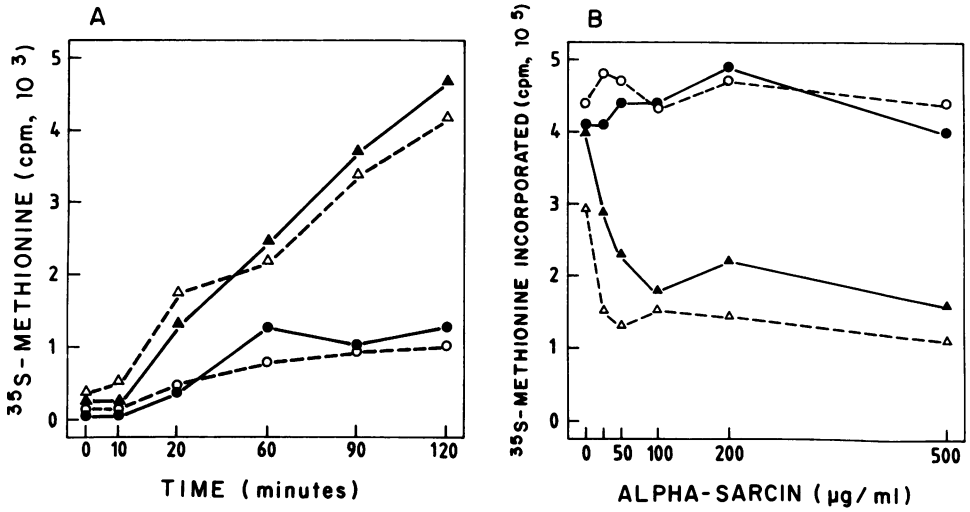

FIG. 5. Effect of carrageenan on (A) entry of HSV-1 into HeLa cells and (B) early cell permeability induced by HSV-1. (A) [^{35}S]methionine-labeled HSV-1 was purified as described in Materials and Methods. Radiolabeled virus at 120,000 cpm was added to HeLa cells in the presence (△) of 100 μg of carrageenan per ml or in the absence (▲) of the polysaccharide. Virus entry into cells at 37°C was measured as described in Materials and Methods. Background of entry was determined by the addition of [^{35}S]methionine-labeled HSV-1 to cells in the absence (●) or presence (○) of the compound at 4°C. (B) HeLa monolayers were infected with HSV-1 at a multiplicity of infection of 100 in the presence of 100 μg of carrageenan per ml and different concentrations of alpha-sarcin. After 1 h of incubation at 37°C, the cell monolayers were washed three times, and 1 h later protein synthesis was estimated as described in Materials and Methods. Symbols: ●, uninfected control cells; ○, uninfected cells treated with carrageenan; ▲, HSV-1-infected control cells; △, HSV-1-infected cells treated with carrageenan.

ized to proteins such as alpha-sarcin (8) or *Pseudomonas* toxin (9). Carrageenan did not inhibit this process (Fig. 5B), further reinforcing the idea that virus attachment and entry are not the steps blocked by sulfated polysaccharides.

To determine whether carrageenan must be present together with HSV-1 virion particles to prevent viral protein synthesis, cells were infected with HSV-1 in the absence or presence of 100 μg of carrageenan per ml. After 0.5 h of incubation, the excess virus and polysaccharide were removed from the culture medium and some cultures were further superinfected with HSV-1. In agreement with previous results, when the cells are infected with HSV-1 in the presence of carrageenan no viral proteins appear even after 18 h postinfection (data not shown). However, if the cells have been pretreated with the compound or with the compound plus herpes virions, there is no inhibition of a subsequent infection by HSV-1.

DISCUSSION

Polysaccharides were found to be potent antiviral agents, but the antiviral effects of these compounds were not pursued perhaps for two reasons: (i) they are high-molecular-weight components, and it is unlikely that they pass the different barriers of the body or even the cell membrane; and (ii) it was thought that they blocked viral attachment, an "unrewarding step in antiviral chemotherapy" (19).

Although it is unlikely that large polysaccharides can penetrate the skin for topical use against certain cutaneous HSV-1 infections, it is possible that small oligosaccharides such as dextran sulfate 5000 pass those barriers easier. In fact, oligosaccharides are found in the blood when rats are fed with oral heparin, suggesting that these compounds are much more difusible in the body than previously thought (12). In agreement with previous findings (17), our studies suggest that antivirally inert polysaccharides such as dextrans become active when sulfate groups are present in the molecule. This opens the possibility of using rather inexpensive oligosaccharides, which are abundant in nature, and converting them by a simple reaction into antiviral compounds. We do not yet know if the presence of other groups in the molecule other than sulfate also confer the biological activity reported in this work.

Polysaccharides are thought to inhibit the very early step of viral replication, i.e., virus attachment to the cell surface (19). In agreement with this idea, our results indicate that viral protein synthesis is prevented by carrageenan, and this inhibition is observed only when the compound is present during virus entry. However, in contrast with the suggestion that sulfated polysaccharides inhibit virus attachment, our findings with labeled virion particles clearly indicate that virions are internalized at control levels, even with concentrations of carrageenan 10 times greater than those necessary to block viral replication. It must be emphasized that the suggestion that sulfated polysaccharides inhibit virus adsorption (20) was based on a rather crude test. Briefly, the experiment consisted of the incubation of cells with viruses in the presence of the polysaccharide followed by the washing away of the inhibitor. Afterwards, the cells were overlaid with agar and incubated to count viral plaques. A reduction in the number of viral plaques was taken as evidence of inhibition of virus attachment. Our results indicating that there is virus internalization but that a subsequent step in viral replication is blocked even if carrageenan is washed out (not shown) are in agreement with these early findings but indicate that their interpretation was not wholly correct.

Since HSV-1 was still able to permeabilize cells to alpha-sarcin, a process thought to be mediated by the permeabilization of pinocytotic vesicles (15), the possibility exists that virions are released from those vesicles even in the presence of the inhibitor. If this were so, those virions would

be either unable to decapsidate or unable to fully express their genomes, since no late viral proteins appear in the presence of carrageenan.

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LITERATURE CITED

1. **Alarcón, B., J. C. Lacal, J. M. Fernández-Sousa, and L. Carrasco.** 1984. Screening for new compounds with antiherpes activity. Antiviral Res. **4**:231–243.
2. **Aspinall, G. O.** 1983. The polysaccharides, vol. 2, p. 1–9. Academic Press, Inc., New York.
3. **Choppin, P. W., and H. J. Eggers.** 1962. Heterogeneity of particles which differ in antibody sensitivity, growth rate, and plaque size. Virology **18**:470–476.
4. **Colter, J. S., M. A. Davies, and J. B. Campbell.** 1964. Studies of three variants of mengo encephalomyelitis virus. II. Inhibition of interaction with L cells by an agar inhibitor and by protamine. Virology **24**:578–585.
5. **De Clercq, E., and M. Luczak.** 1976. Antiviral activity of carbopol, a cross-linked polycarboxylate. Arch. Virol. **52**: 151–158.
6. **De Somer, P., E. De Clercq, A. Billiau, E. Schonne, and M. Claesen.** 1968. Antiviral activity of polyacrylic and polymethacrylic acids. II. Mode of action in vivo. J. Virol. **2**:886–893.
7. **Farquhar, C. F., and A. G. Dickinson.** 1986. Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. J. Gen. Virol. **67**:463–473.
8. **Fernández-Puentes, C., and L. Carrasco.** 1980. Viral infection permeabilizes mammalian cells to protein toxins. Cell **20**: 769–775.
9. **Fitzgerald, D. J., R. Padmanabhan, I. Pastan, and M. C. Willingham.** 1983. Adenovirus-induced release of epidermal growth factor and pseudomonas toxin into the cytosol of KB cells during receptor-mediated endocytosis. Cell **32**:607–617.
10. **Folkman, J., R. Langer, R. J. Linhardt, C. Haudenschild, and S. Taylor.** 1983. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science **221**:719–725.
11. **Hochberg, E., and Y. Becker.** 1968. Adsorption, penetration, and uncoating of herpes simplex virus. J. Gen. Virol. **2**:231–241.
12. **Larsen, A. K., D. P. Lund, R. Larger, and J. Folkman.** 1986. Oral heparin results in the appearance of heparin fragments in the plasma of rats. Proc. Natl. Acad. Sci. USA **83**:2964–2968.
13. **Liebhaber, H., and K. K. Takemoto.** 1963. The basis for the size differences in plaques produced by variants of encephalomyocarditis (EMC) virus. Virology **20**:559–566.
14. **Merigan, T. C., and M. S. Finkelstein.** 1968. Interferon-stimulating and in vivo antiviral effects of various synthetic anionic polymers. Virology **35**:363–374.
15. **Muñoz, A., J. L. Castrillo, and L. Carrasco.** 1985. Modification of membrane permeability during Semliki Forest virus infection. Virology **146**:203–212.
16. **Nahmias, A. J., and S. Kibrick.** 1964. Inhibitory effect of heparin on herpes simplex virus. J. Bacteriol. **87**:1060–1066.
17. **Nahmias, A. J., S. Kibrick, and P. Bernfeld.** 1964. Effect of synthetic and biological polyanions on herpes simplex virus. Proc. Soc. Exp. Biol. Med. **115**:993–996.
18. **Rees, D. A.** 1969. Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. Adv. Carbohydr. Chem. Biochem. **24**:267–332.
19. **Shannon, W. M.** 1984. Mechanisms of action and pharmacology: chemical agents, p. 55–121. *In* G. J. Galasso, T. C. Merigan, and R. A. Buchanon (ed.), Antiviral agents and viral diseases of man. Raven Press, New York.
20. **Takemoto, K. K.** 1966. Plaque mutants of animal viruses. Prog. Med. Virol. **8**:314–348.
21. **Takemoto, K. K., and P. Fabisch.** 1964. Inhibition of herpes virus by natural and synthetic acid polysaccharides. Proc. Natl. Acad. Sci. USA **116**:140–144.
22. **Takemoto, K. K., and H. Liebhaber.** 1961. Virus polysaccharide interactions. I. An agar polysaccharide determining plaque morphology of EMC virus. Virology **14**:456–462.
23. **Takemoto, K. K., and H. Liebhaber.** 1962. Virus polysaccharide interactions. II. Enhancement of plaque formation and the detection of variants of poliovirus with dextran sulfate. Virology **17**:499–501.
24. **Vaheri, A., and K. Cantell.** 1963. The effect of heparin on herpes simplex virus. Virology **21**:661–662.
25. **Wallis, C., and J. L. Melnick.** 1968. Mechanism of enhancement of virus plaques by cationic polymers. J. Virol. **2**:267–274.